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CONTENTS/SUMMARIES

Fluorogenic and Chromogenic Substrates Used in Bacterial Diagnostics. Mohammed Manafi, Wolfgang Kneifel, and Shoshana Bascomb 335–348

Summary: Methods based on the application of chromogenic and fluorogenic substrates enable specific and rapid detection of a variety of bacterial enzymatic activities. By using these techniques, enzymatic reactions can be examined simultaneously or individually, either directly on the isolation plate or in cell suspensions. For this purpose, various testing principles and test kits for clinical and food microbiology have been introduced successfully during the last few years. In this paper we present a survey of different enzymes of microbial origin that are utilized for microbiological identification and differentiation and the corresponding methods. Particular emphasis is given to the examination of Escherichia coli and the description of the different techniques as used in routine analysis.

Aromatic Amino Acid Biosynthesis in the Yeast *Saccharomyces cerevisiae*: a Model System for the Regulation of a Eukaryotic Biosynthetic Pathway. Gerhard H. Braus 349–370

Summary: This review focuses on the gene-enzyme relationships and the regulation of different levels of the aromatic amino acid biosynthetic pathway in a simple eukaryotic system, the unicellular yeast Saccharomyces cerevisiae. Most reactions of this branched pathway are common to all organisms which are able to synthesize tryptophan, phenylalanine, and tyrosine. The current knowledge about the two main control mechanisms of the yeast aromatic amino acid biosynthesis is reviewed. (i) At the transcriptional level, most structural genes are regulated by the transcriptional activator GCN4, the regulator of the general amino acid control network, which couples transcriptional derepression to amino acid starvation of numerous structural genes in multiple amino acid biosynthetic pathways. (ii) At the enzyme level, the carbon flow is controlled mainly by modulating the enzyme activities at the first step of the pathway and at the branch points by feedback action of the three aromatic amino acid end products. Implications of these findings for the relationship of S. cerevisiae to prokaryotic as well as to higher eukaryotic organisms and for general regulatory mechanisms occurring in a living cell such as initiation of transcription, enzyme regulation, and the regulation of a metabolic branch point are discussed.

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Control Site Location and Transcriptional Regulation in *Escherichia Coli*. Julio Collado-Vides, Boris Magasanik, and Jay D. Gralla 371–394

Summary: The regulatory regions for 119 Escherichia coli promoters have been analyzed, and the locations of the regulatory sites have been cataloged. The following observations emerge. (i) More than 95% of promoters are coregulated with at least one other promoter. (ii) Virtually all sigma 70 promoters contain at least one regulatory site in a proximal position, touching at least position −65 with respect to the start point of transcription. There are not yet clear examples of upstream regulation in the absence of a proximal site. (iii) Operators within regulons appear in very variable proximal positions. By contrast, the proximal activation sites of regulons are much more fixed. (iv) There is a forbidden zone for activation elements downstream from approximately position −20 with respect to the start of transcription. By contrast, operators can occur throughout the proximal region. When activation elements appear in the forbidden zone, they repress. These latter examples usually involve autoregulation. (v) Approximately 40% of repressible promoters contain operator duplications. These occur either in certain regulons where duplication appears to be a requirement for repressor action or in promoters subject to complex regulation. (vi) Remote operator duplications occur in approximately 10% of repressible promoters. They generally appear when a multiple promoter region is coregulated by cyclic AMP receptor protein. (vii) Sigma 54 promoters do not require proximal or precisely positioned activator elements and are not generally subject to negative regulation. Rationales are presented for all of the above observations.

Genetic Competence in *Bacillus subtilis*. David Dubnau 395–424

Summary: Genetic competence may be defined as a physiological state enabling a bacterial culture to bind and take up high-molecular-weight exogenous DNA (transformation). In Bacillus subtilis, competence develops postexponentially and only in certain media. In addition, only a minority of the cells in a competent culture become competent, and these are physiologically distinct. Thus, competence is subject to three regulatory modalities: growth stage specific, nutritionally responsive, and cell type specific. This review summarizes the present state of knowledge concerning competence in B. subtilis. The study of genes required for transformability has permitted their classification into two broad categories. Late competence genes are expressed under competence control and specify products required for the binding, uptake, and processing of transforming DNA. Regulatory genes specify products that are needed for the expression of the late genes. Several of the late competence gene products have been shown to be membrane localized, and others are predicted to be membrane associated on the basis of amino acid sequence data. Several of these predicted protein sequences show a striking resemblance to gene products that are involved in the export and/or assembly of extracellular proteins and structures in gram-negative organisms. This observation is consistent with the idea that the late products are directly involved in transport of DNA and is equally consistent with the notion that they play a morphogenetic role in the assembly of a transport apparatus. The competence regulatory apparatus constitutes an elaborate signal transduction system that senses and interprets environmental information and passes this information to the competence-specific transcriptional machinery. Many of the regulatory gene products have been identified and partially characterized, and their interactions have been studied genetically and in some cases biochemically as well. These include several histidine kinase and response regulator members of the bacterial two-component signal transduction machinery, as well as a number of known transcriptionally active proteins. Results of genetic studies are consistent with the notion that the regulatory proteins interact in a hierarchical way to make up a regulatory pathway, and it is possible to propose a provisional scheme for the organization of this pathway. It is remarkable that almost all of the regulatory gene products appear to play roles in the control of various forms of postexponential expression in addition to competence, e.g., sporulation, degradative-enzyme production, motility, and antibiotic production. This has led to the notion of a signal transduction network which transduces environmental information to determine the levels and timing of expression of the ultimate products characteristic of each of these systems.

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***Bacillus sphaericus* as a Mosquito Pathogen: Properties of the Organism and Its Toxins.** Paul Baumann, Marta A. Clark, Linda Baumann, and Andrew H. Broadwell 425–436

Summary: In the course of sporulation, Bacillus sphaericus produces an inclusion body which is toxic to a variety of mosquito larvae. In this review we discuss the general biology of this species and concentrate on the genetics and physiology of toxin production and its processing in the midgut of the larval host. The larvicide of B. sphaericus is unique in that it consists of two proteins of 51 and 42 kDa, both of which are required for toxicity to mosquito larvae. There is a low level of sequence similarity between these two proteins, which differ in their sequences from all the other known insecticidal proteins of Bacillus thuringiensis. Within the midgut the 51- and 42-kDa proteins are processed to proteins of 43 and 39 kDa, respectively. The conversion of the 42-kDa protein to a 39-kDa protein results in a major increase in toxicity; the significance of the processing of the 51-kDa protein is not known. In contrast to the results with mosquito larvae, the 39-kDa protein is alone toxic for mosquito-derived tissue culture-grown cells, and this toxicity is not affected by the 51-kDa protein or its derivative, the 43-kDa protein. Comparisons of larvae from species which differ in their susceptibility to the B. sphaericus toxin indicate that the probable difference resides in the nature of the target sites of the epithelial midgut cells and not in uptake or processing of the toxin. A similar conclusion is derived from experiments involving tissue culture-grown cells from mosquito species which differ in their susceptibility to the B. sphaericus toxin.

Colicin V Virulence Plasmids. Virginia L. Waters and Jorge H. Crosa 437–450

Summary: ColV plasmids are a heterogeneous group of IncFI plasmids which encode virulence-related properties such as the aerobactin iron uptake system, increased serum survival, and resistance to phagocytosis. These plasmids have been found in invasive strains of Escherichia coli which infect vertebrate hosts including humans and livestock. Colicin V was the first colicin to be identified, in 1925, but not until the field experienced a renewed interest has the mechanism of colicin V activity been explored. As encoded by ColV plasmid pColV-K30, the aerobactin iron uptake system has been extensively investigated, but other ColV-encoded phenotypes remain largely uncharacterized. Restriction enzyme mapping of the 144-kb pColV-K30 and of the 80-kb pColV-B188 has facilitated systematic study, so that questions can be addressed by a molecular and comparative approach regarding the contributions of individual factors and plasmids to the virulence of host E. coli in model systems. The family of large ColV plasmids could be analogous to other families of large virulence plasmids, and insights gained from studying these plasmids should contribute to our understanding of cross-genetic interactions and the role of large plasmids in bacterial pathogenesis.

DNA Methylation and Gene Expression. Aharon Razin and Howard Cedar..... 451–458

Summary: A large body of evidence demonstrates that DNA methylation plays a role in gene regulation in animal cells. Not only is there a correlation between gene transcription and undermethylation, but also transfection experiments clearly show that the presence of methyl moieties inhibits gene expression in vivo. Furthermore, gene activation can be induced by treatment of cells with 5-azacytidine, a potent demethylating agent. Methylation appears to influence gene expression by affecting the interactions with DNA of both chromatin proteins and specific transcription factors. Although methylation patterns are very stable in somatic cells, the early embryo is characterized by large alterations in DNA modification. New methodologies are now becoming available for studying methylation at this stage and in the germ line. During development, tissue-specific genes undergo demethylation in their tissue of expression. In tissue culture cells this process is highly specific and appears to involve an active mechanism which takes place in the absence of DNA replication. The X chromosome undergoes inactivation during development; this is accompanied by de novo methyla-

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tion, which appears necessary to stably maintain its silent state. As opposed to the programmed changes in DNA methylation which occur in vivo, immortalized tissue culture cells demonstrate alterations in DNA modification which take place over a long time scale and which appear to be the result of selective pressures present during the growth of these cells in culture.

Control of Cyclic Chromosome Replication in *Escherichia coli* Hans Bremer and Gordon Churchward..... 459–475

Summary: The biochemical basis for cyclic initiation of bacterial chromosome replication is reviewed to define the processes involved and to focus on the putative oscillator mechanism which generates the replication clock. The properties required for a functional oscillator are defined, and their implications are discussed. We show that positive control models, but not negative ones, can explain cyclic initiation. In particular, the widely accepted idea that DnaA protein controls the timing of initiation is examined in detail. Our analysis indicates that DnaA protein is not involved in the oscillator mechanism. We conclude that the generation of a signal leading to cyclic initiation is separate from the initiation process itself and propose a heuristic model to focus attention on possible oscillator mechanisms.

***Listeria monocytogenes*, a Food-Borne Pathogen.** J. M. Farber and P. I. Peterkin..... 476–511

Summary: The gram-positive bacterium Listeria monocytogenes is an ubiquitous, intracellular pathogen which has been implicated within the past decade as the causative organism in several outbreaks of foodborne disease. Listeriosis, with a mortality rate of about 24%, is found mainly among pregnant women, their fetuses, and immunocompromised persons, with symptoms of abortion, neonatal death, septicemia, and meningitis. Epidemiological investigations can make use of strain-typing procedures such as DNA restriction enzyme analysis or electrophoretic enzyme typing. The organism has a multifactorial virulence system, with the thiol-activated hemolysin, listeriolysin O, being identified as playing a crucial role in the organism's ability to multiply within host phagocytic cells and to spread from cell to cell. The organism occurs widely in food, with the highest incidences being found in meat, poultry, and seafood products. Improved methods for detecting and enumerating the organism in foodstuffs are now available, including those based on the use of monoclonal antibodies, DNA probes, or the polymerase chain reaction. As knowledge of the molecular and applied biology of L. monocytogenes increases, progress can be made in the prevention and control of human infection.

Relationship of Eukaryotic DNA Replication to Committed Gene Expression: General Theory for Gene Control Luis P. Villarreal..... 512–542

Summary: The historic arguments for the participation of eukaryotic DNA replication in the control of gene expression are reconsidered along with more recent evidence. An earlier view in which gene commitment was achieved with stable chromatin structures which required DNA replication to reset expression potential (D. D. Brown, Cell 37:359–365, 1984) is further considered. The participation of nonspecific stable repressor of gene activity (histones and other chromatin proteins), as previously proposed, is reexamined. The possible function of positive trans-acting factors is now further developed by considering evidence from DNA virus models. It is proposed that these positive factors act to control the initiation of replicon-specific DNA synthesis in the S phase (early or late replication timing). Stable chromatin assembles during replication into potentially active (early S) or inactive (late S) states with prevailing trans-acting factors (early) or repressing factors (late) and may asymmetrically commit daughter templates. This suggests logical schemes for programming differentiation based on replicons and trans-acting initiators. This proposal requires that DNA replication precede major changes in gene commitment. Prior evidence against a role for DNA

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replication during terminal differentiation is reexamined along with other results from terminal differentiation of lower eukaryotes. This leads to a proposal that DNA replication may yet underlie terminal gene commitment, but that for it to do so there must exist two distinct modes of replication control. In one mode (mitotic replication) replicon initiation is tightly linked to the cell cycle, whereas the other mode (terminal replication) initiation is not cell cycle restricted, is replicon specific, and can lead to a terminally differentiated state. Aberrant control of mitotic and terminal modes of DNA replication may underlie the transformed state. Implications of a replicon basis for chromatin structure-function and the evolution of metazoan organisms are considered.